Biochemical and Biophysical Research Communications 453 (2014) 419–424

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Blood–brain barrier dysfunction in mice induced by lipopolysaccharide is attenuated by dapsone

Ting Zhou [a,1], Lei Zhao [a,1], Rui Zhan [a], Qihua He [b], Yawei Tong [a], Xiaosheng Tian [a], Hecheng Wang [a], Tao Zhang [a], Yaoyun Fu [a], Yang Sun [a], Feng Xu [c], Xiangyang Guo [d,*], Dongsheng Fan [d], Hongbin Han [d,*], Dehua Chui [a,d,*]

[a] Neuroscience Research Institute and Department of Neurobiology, Key Laboratory for Neuroscience, Ministry of Education and Ministry of Public Health, Health Science Center, Peking University, Beijing, China
[b] Center of Medical and Health Analysis, Peking University, Beijing, China
[c] Department of Natural Medicines, School of Pharmaceutical Sciences, Peking University, Beijing, China
[d] Peking University Third Hospital, Beijing, China

ARTICLE INFO

Article history:
Received 18 September 2014
Available online 28 September 2014

Keywords:
Dapsone
Blood–brain barrier integrity
Lipopolysaccharide

ABSTRACT

Blood–brain barrier (BBB) dysfunction is a key event in the development of many central nervous system (CNS) diseases, such as septic encephalopathy and stroke. 4,4′-Diaminodiphenylsulfone (DDS, Dapsone) has displayed neuroprotective effect, but whether DDS has protective role on BBB integrity is not clear. This study was designed to examine the effect of DDS on lipopolysaccharide (LPS)-induced BBB disruption and oxidative stress in brain vessels. Using in vivo multiphoton imaging, we found that DDS administration significantly restored BBB integrity compromised by LPS. DDS also increased the expression of tight junction proteins occludin, zona occludens-1 (ZO-1) and claudin-5 in brain vessels. Level of reactive oxygen species (ROS) was reduced by DDS treatment, which may due to decreased nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity and NOX2 expression. Our results showed that LPS-induced BBB dysfunction could be attenuated by DDS, indicated that DDS has a therapeutic potential for treating CNS infection and other BBB related diseases.

1. Introduction

The blood–brain barrier (BBB) is comprised of brain microvascular endothelial cells and regulates the permeation of molecules between the peripheral circulation and the central nervous system (CNS) [1]. The main structures responsible for the maintenance of the integrity and function of BBB are the intercellular tight junction proteins [2]. BBB dysfunction is considered to be an early and significant event in the pathogenesis of a variety of CNS diseases [3–5], so the protection of BBB integrity is regarded as one of the important issues for the treatment of many cerebral diseases [6].

Dysfunction of BBB can be induced by various extrinsic or intrinsic stimuli [7]. Such as sepsis, during which lipopolysaccharide (LPS) is released into circulation, promoting the generation of reactive oxygen species (ROS) in BBB [8]. A major sources of ROS during BBB dysfunction are nicotinamide adenine dinucleotide phosphate (NADPH) oxidases [9]. There are several members in the NADPH oxidase family, among which NOX2 containing NADPH oxidase is highly expressed in cerebral endothelium [10]. Reducing expression of NOX2 can protect mice from a variety of stimuli that produce cerebrovascular dysfunction [11–13].

4,4′-Diaminodiphenylsulfone (DDS, Dapsone) is currently used to treat leprosy [14] and is known to possess neuroprotective effect against ischemia, spinal cord injury and other brain damage [15–19]. One of its modes of action is anti-oxidant [17,20]. DDS reduces ROS generation in *Caenorhabditis elegans* thus extends its lifespan [20]. In non-phagocytic human diploid fibroblasts and a mouse lung injury model, DDS also suppresses ROS production by inhibiting the NOX system [21,22]. As BBB integrity is disrupted during the pathological process of many CNS disease, especially stroke, and DDS treatment reduces the infarction volume in rat ischemia model [18], so we hypothesize that DDS may have a protective role on BBB integrity, and may be via reducing oxidative stress status.

The present study focus on effect of DDS on LPS-induced BBB disruption in mice. Our data showed that DDS inhibited LPS-induced BBB dysfunction, restored expression of tight junction, as

* Corresponding author at: Neuroscience Research Institute, Peking University Health Science Center, 38 Xueyuan Road, Hai Dian District, 100191 Beijing, China.
E-mail address: dchui@bjmu.edu.cn (D. Chui).
[1] These authors contributed equally to this paper.

http://dx.doi.org/10.1016/j.bbrc.2014.09.093
0006-291X/

well as decreased ROS level, NOX2 expression and NADPH oxidase activity in brain vessels, indicated that DDS can protect the integrity of BBB during septic encephalopathy.

2. Materials and methods

2.1. Animals and drug administration

Female C57BL/6J mice (20–25 g body weight, 3 month-old) were housed under standard conditions in conventional cages and kept on standard chow diet and water ad libitum with 12-h light and dark cycles. The experiments were designed as follows: LPS (Sigma, St. Louis, MO) was dissolved in saline solution, and DDS (Sigma, St. Louis, MO) was dissolved in 4.5% polyethylene glycol in saline solution (PEG) [16]. Mice in control group were intraperitoneal (i.p.) injected with 100 μl saline solution, followed by 100 μl PEG after 30 min; mice in LPS group were injected with 1 mg/kg LPS (the volume was adjusted to 100 μl), followed by 100 μl PEG after 30 min; mice in LPS + DDS group were injected with 0.5, 2 or 5 mg/kg DDS (the volume was adjusted to 100 μl) 30 min after LPS administration. Assessments were performed 24 h after DDS (or PEG) injection. At least 6 mice per group were studied. All procedures were approved by the Animal Care Committee of Peking University Health Science Center in China.

2.2. Multiphoton in vivo microscopy analysis

In vivo multiphoton imaging was performed as previously described [3,23]. 24 h after LPS administration, mice were anesthetized and the cranium was firmly secured in a stereotaxic frame. A square cranial window was opened with a high-speed drill. Tetramethylrhodamine (TMR) -conjugated dextran (40 kDa, 0.1 mL of 10 mg/ml, Invitrogen, Carlsbad, CA) was injected via the tail vein. At the end of the experiment mice were killed by decapitation and brains were harvested, frozen and conserved at −80 °C until use.

In vivo images were acquired using a multiphoton microscope (Leica TCS SP5 MP, Chicago, IL) with 850 nm excitation and 20×/1.0 water immersion objective, 2 mm working distance. Once the area of interest was defined, 200 μm-thick stacks in the *Z*-axis (5 μm steps) were obtained with the Leica ASF software. The relative fluorescence intensity across cross-section of vessels was analyzed with ImageJ (NIH) software [24].

2.3. Isolation of brain capillaries

Brain capillaries were isolated using dextran gradient centrifugation as described [3,23]. The cortex and hippocampus were carefully dissected and the meninges were removed in ice-cold PBS containing 2% fetal bovine serum (FBS). The brain was homogenized and dextran (70 kDa, Pharmacia) was added to a concentration of 16%. The samples were then centrifuged at 6000*g* for 15 min. The capillary pellet located at the bottom of the tubes was collected and sequentially filtered through a 100 and 45 μm cell strainer. The capillaries remaining on top of the 45 μm cell strainer were collected in PBS.

2.4. Western blot analysis

Isolated brain capillaries were lysed in RIPA buffer, and western blot analysis was carried out as previously described [23].The protein concentration of each homogenate was determined using a BCA kit (Pierce). Extracts (60 μg of protein) were subjected to electrophoresis, and separated proteins were transferred onto PVDF membranes, which were then immunostained with the following primary antibodies against occludin (1:500, Invitrogen, Carlsbad, CA), claudin-5 (1:500, Abcam, San Diego, CA), ZO-1 (1:500, Invitrogen, Carlsbad, CA), NOX2 (1:3000, Santa Cruz Biotechnology, Santa Cruz, CA) and β-actin (1:5000, Sigma, St Louis, MO). The membranes were incubated with peroxidase-conjugated secondary antibodies, and immunoreactive bands were visualized with an ECL system.

2.5. Cerebrovascular oxidative stress assessment

Cerebrovascular oxidative stress was determined as described [23]. Isolated brain capillaries were incubated for 30 min in darkness in the presence of 10 μM dichlorofluorescein diacetate. Fluorescence intensity (λexc 485 nm, λem 530 nm) was read in a flexstation 3 microplate reader (molecular devices), normalized for protein concentration.

2.6. NADPH oxidase activity assay

NADPH oxidase activity was measured as described [11]. Isolated brain vessels were homogenized in Krebs–Ringer phosphate buffer at pH 7.4 (120 mmol/L NaCl, 4.8 mmol/L KCl, 1.2 mmol/L MgSO4, 2.2 mmol/L CaCl2, 0.1 mol/L phosphate buffer) with phenylmethylsulfonyl fluoride and protease inhibitor cocktail (Roche). 5 μM lucigenin were added to tissue extracts. The reaction was initiated by adding 10 μL of NADPH solution to the final concentration 100 μM. For each sample, 30 s integrated chemiluminescence was measured and repeated for 5 times. After measurement, the samples were recollected and protein quantification was calculated using a BCA kit. Respective background counts were subtracted, and chemiluminescence was expressed in relative light units per microgram protein.

2.7. Statistical analysis

All data in the text and figures are expressed as mean ± SEM of at least three independent experiments [23,25]. A one-way analysis of variance (ANOVA) followed by Dunnett's or Tukey–Kramer's post hoc tests was performed to compare groups. Mean values were considered significantly different at $p < 0.05$, $p < 0.01$ or $p < 0.001$.

3. Results

3.1. BBB dysfunction induced by LPS was attenuated by DDS

To determine whether DDS has protective effect on BBB integrity, we examined the in vivo BBB permeability for TMR-dextran with multiphoton microscopy. We found that mice in control group displayed intact BBB, while LPS treatment increased BBB permeability significantly (Fig. 1A), the relative fluorescence intensity across a cross-section of vessels increased to 6.4 ± 0.7-fold in LPS group compared with control ($p < 0.01$) (Fig. 1B). Administration of 5 mg/kg DDS significantly inhibited LPS-induced BBB leakage (Fig. 1A), and the relative fluorescence intensity decreased to 1.6 ± 0.5-fold compared with control ($p < 0.01$) (Fig. 1B), suggested that DDS attenuated BBB injury during infection.

3.2. Expression of tight junction proteins was restored by DDS

BBB integrity is maintained by the presence of tight junction proteins, so we assessed expression of tight junction proteins ZO-1, occludin and claudin-5 in brain vessels of mice with western blotting. Results showed that LPS significantly decreased expression of ZO-1, occludin and claudin-5 to 0.5 ± 0.1-fold ($p < 0.001$),